



The Potency of Soft Coral Sarcophyton in Krakatau Seas as Cytotoxic Test and its Relation Towards Water Acidification

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ABSTRACT

Soft coral is one of the marine organisms that produce secondary metabolites materials and has potency as anticancer. There are many studies about bioactive compounds in soft coral but still lack of information about the potency of bioactive in soft coral that lives in acidic environment. The objectives of this study are to determine the characteristic of acidification in Krakatau seas and to assess the potency of cytotoxic activity from soft coral *Sarcophyton*. Sampling was conducted in three locations, Legon Tuo, Legon Cabe, and Umang-umang Island, to collect the samples and measure water quality. Cytotoxic was tested using MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinon) toward MCF-7 cancer cell line to measure the percentage of growth inhibition. Data were analyzed using ANOVA, post hoc analysis, and multivariate analysis. Water quality in Legon Tuo and Legon Cabe have different characteristics compared to Umang-umang Island. Legon Tuo and Legon Cabe are suitable as acidification site, while Umang-umang Island as the reference site. The characteristic of Krakatau seas, especially in Legon Tuo and Legon Cabe, was categorized in low acidification. In the cytotoxic activity, Legon Tuo produces high active cell line with 74.55 % of growth inhibition, while Umang-umang Island produces at 21.57 %. Soft coral *Sarcophyton* from Legon Cabe has actively inhibited the growth of breast cancer cell (MCF-7) than soft coral from Umang-umang Island.

Keywords: acidification, cytotoxic, Krakatau seas, MCF-7 cell, *Sarcophyton*

ABSTRAK

Karang lunak merupakan salah satu organisme yang memproduksi bahan metabolit sekunder dan berpotensi sebagai anti kanker. Banyak penelitian mengenai kandungan bioaktif di karang lunak, tetapi sedikit informasi mengenai potensi kandungan bioaktif karang lunak yang hidup di kondisi asam. Tujuan dari penelitian ini adalah untuk menentukan karakteristik asidifikasi di perairan Krakatau dan mengukur potensi aktifitas sitotoksik dari karang lunak *Sarcophyton*. Pengambilan sampel karang lunak dan sampel air dilakukan di tiga lokasi, yaitu Legon Tuo, Legon Cabe, dan Pulau Umang-umang. Uji sitotoksik dilakukan menggunakan metode MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinon) terhadap sel kanker MCF-7 untuk mengukur persentase hambatan pertumbuhan pada sel tersebut. Kualitas air di Legon Tuo dan Legon Cabe memiliki karakteristik berbeda dibandingkan dengan Pulau Umang-umang. Legon Tuo dan Legon Cabe cocok sebagai perairan terasidifikasi, sedangkan Pulau Umang-umang sebagai perairan referensi. Karakter perairan Krakatau, terutama di Legon Tuo dan Legon Cabe dikategorikan sebagai perairan dengan tingkat asidifikasi rendah. Dalam hal aktifitas sitotoksik, sampel di Legon Tuo memproduksi sel aktif yang tinggi dengan persentase hambatan pertumbuhan hingga 74.55 %, sementara sampel di Pulau Umang-umang memproduksi sebesar 21.57 %. Karang lunak *Sarcophyton* dari Legon Cabe secara aktif menghambat pertumbuhan sel kanker daripada sampel dari Pulau Umang-umang.

Kata kunci: asidifikasi, Perairan Krakatau, *Sarcophyton*, Sel MCF-7, sitotoksik

1. Introduction

Krakatau islands are formed from a big eruption activity and have built new four small islands at the same place, named Rakata Island, Sertung Island, Panjang Island, and Anak Krakatau Volcano that have continued eruption activity. Putra et al., (2014) stated that the succession process which was happened around Anak Krakatau volcano was categorized as the beginning state of the settlement, while in Rakata and Sertung Island have indicated with diversification in the coral community from opportunist and pioneer species (*Pocillopora* and *Seriatopora*).

This area with volcanic activity certainly produces natural carbon dioxide that can lead to decreasing in pH (acidification). This condition can be a reference to the biological changing marine organism that lives in lower pH habitat (UNEP, 2010). Acidification condition, caused by natural carbon dioxide, is used to assess in the long-term effect of acidification on the benthic organism and its habitat (Hall-Spencer et al., 2008). Furthermore, the acidification process has negative impact on coral reef structure. With decreasing in pH, it triggered in declining of calcification process, polyp growth, and zooxanthellae cell density on Scleractinian corals (Januar et al., 2016a). Environment pressures affected soft coral to produce the natural bioactive compound that used to keep its existence in the ecosystem.

Soft coral is the wealthy source for producing the bioactive compounds, such as terpenoid, steroid, and steroid glycoside. Terpenoid compound is the dominant compound in soft coral (Harper et al., 2001). Soft coral, such as *Sinularia*, *Lobophytum*, *Sarcophyton*, *Nephtea*, and *Xenia*, produce bioactive compound because of secondary metabolite activity (Rozirwan et al., 2014). Soft coral belonging to genus *Sarcophyton* has a wealth of secondary metabolites. Terpenes are the most frequently encountered and exhibited a wide range of biological features ranging from antifeedant, anti-inflammatory, antiviral, antifouling, ichthyo-toxic, cytotoxic, to

neuroprotective activities (Liang and Guo, 2013). Secondary metabolite compounds have the potency to be used as material anticancer (Russo et al., 2011; Cooper and Yao, 2012; Dobretsov et al., 2016).

The study in finding potency bioactive compound as anticancer is important to do due to cancer becomes the highest cause of death in the world. In 2012, 4.3 million people died because of cancer and were predicted to increase until 44% from 2012 to 2030 (WHO, 2015), as well as predicted in 2013 causing more than 8 million people died (Eniu et al., 2016). Cancer becomes the main health problem even in the developed country (Sawadogo et al., 2015).

In Indonesia, studies about exploration in secondary metabolite from the marine organism which live in extreme condition have been conducted, but it still lacks data. Unfortunately, there is no study about the potency of anticancer from soft coral *Sarcophyton* in Krakatau seas. Therefore, the objectives of this study are to determine the characteristic of acidification in Krakatau seas and to assess the potency of bioactive activity from soft coral *Sarcophyton*.

2. Materials and Methods

2.1. Sampling

Sampling was conducted on April 15-16, 2016 in three different study site (Figure 1) at about 4-5 meters depth in Legon Cabe (6,1444° S; 105,4261° E), Legon Tuo (6,1468° S; 105,4624° E), and Umang-umang Island (5,9299° S; 105,5140° E). Legon Cabe and Legon Tuo located close to Anak Krakatau volcano and were assumed have acidification activity, while Umang-umang Island located far away from the volcano and was assumed have no acidification activity. Furthermore, we called Legon Cabe and Legon Tuo as acidification site, while Umang-umang island as the reference site.

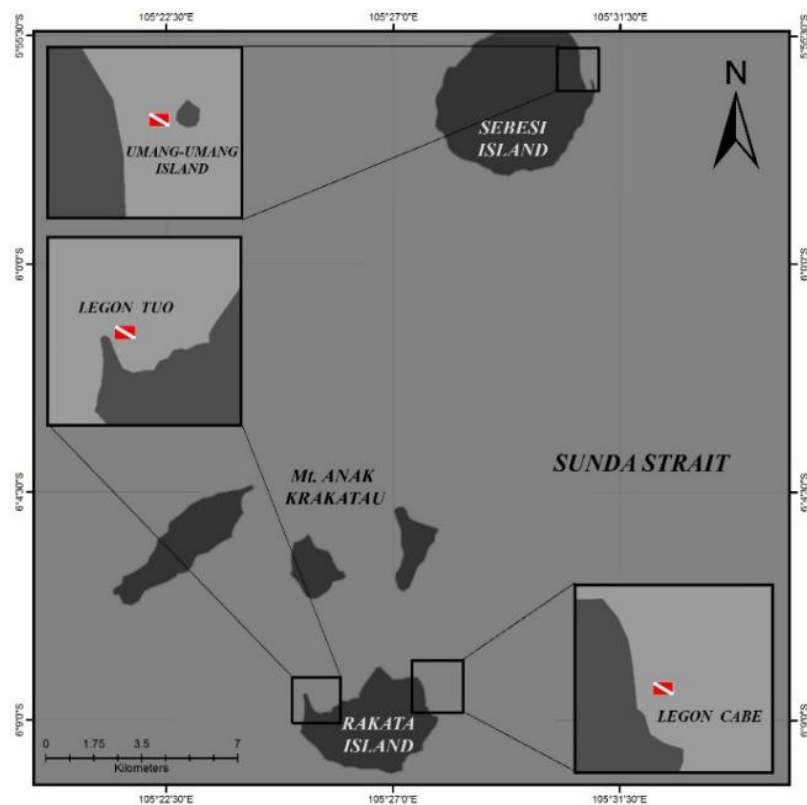


Figure 1. Sampling location in Krakatau, Sunda Strait

During sampling, we directly measured water quality (dissolve oxygen (DO), pH, salinity, phosphate, nitrate, and ammonia) and recorded the position using GPS (Global Positioning System). We measured DO and pH using a multimeter (HACH HQ40d) and salinity using salinometer (EUTECH Instruments SALT6+). While phosphate, nitrate, and ammonia were measured by using colorimeter (HACH DR/850 portable colorimeter). After got the value of water quality by direct measurement, we used those values to calculate carbonate chemistry concentration (alkalinity, the atmospheric partial pressure of carbon dioxide, calcite, and aragonite) in each site using CO₂SYS v2.1 (Pierrot et al., 2006).

Some part of *Sarcophyton* was cut off from the main individual, and then immediately preserved into spuncum collection tube with 20 mL ethanol (concentration 96%). Then we stored the sample in the cool box to keep the temperature stable and avoided from damaged. This procedure is important because it can keep the samples in good condition until we do the next process in Laboratory. We collected five samples in total of *Sarcophyton*, two samples from Legon Cabe and the remains from Umang-umang Island.

2.2. Sampling extraction and cytotoxic test

We conducted sample extraction according to Wikanta et al., (2005). After sample already solved with ethanol, then freeze-dry the sample until dry. Then we took 1 gram sample and macerated it with 1 mL ethanol. After 72 h of maceration procedure, filter sample using filter disc 0.45 µm. Put sample into Eppendorf tube 1.5 mL and then dry it using concentrator with temperature -64 °C and pressure 1 mbar. This procedure generated dry powder for the cytotoxic test.

The cytotoxic test was performed using MCF-7 (breast cancer) cell line according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazo-linon) method (Zachary, 2003). Lee at al., (2015) stated that MCF-7 cell line mostly used by scientists in the world due to the results have had a fundamental impact upon breast cancer research and patient outcomes. To find the percentage of growth inhibition of cancer cell lines, we calculated as shown in equation 1 wherein the absorbance of A (cancer cell control), B (sample), C (sample control), and D (medium control). We got the absorbance value using microplate reader (DYNEX microplate reader) with 595 nm absorbance. We referred to Januar et al., (2016b) for more detail of the cytotoxic test procedure.

$$\text{inhibition (\%)} = \frac{(A-D)(B-C)}{(A-D)} \dots\dots\dots (1)$$

2.3. Data analysis

Water quality data were analyzed by using analysis of variance (ANOVA) and Bonferroni test for the posterior comparisons (post hoc analysis) to find the possibility of the difference between the groups. We conducted the statistical analysis using statistical computing software R (version 3.4.1). In other cases, we analysed the water quality data using Canonical Discriminant Analysis (CDA) and used statistical software PAST (version 3). CDA is a multivariate technique that can be used to determine the relationship between a categorical variable and a group of independent variables (Zhao and Maclean, 2000).

3. Results and Discussion

3.1. Water quality condition in Krakatau Seas

We have measured six parameters directly in the field and another four parameters based on CO₂SYS calculation. The results have been shown at **Table 1**. According to Table 1, ANOVA revealed significant differences between parameters of water quality with study site. Almost all of the parameters we could find the significant difference ($p < 0.05$), but we could not find any significant difference in

nitrate. These results indicate that most of the parameters have the effect in determining the characteristic of the study site.

According to further tests on each parameter, there was an alphabet marked difference indicating significant different ($p < 0.05$). Most of the parameters in Legon Tuo and Legon Cabe have significantly different from Umang-umang Island. These results indicated that the characteristics of water quality in Legon Tuo and Legon Cabe have similarity due to the positions of both study sites, which are near to Anak Krakatau Volcano.

For more detail to determine which parameters have the relationship to study site, **Figure 2** showed the result of Canonical Discriminant Analysis (CDA). It showed that there was a dominant parameter in each study site which showed the character itself. For instance, Legon Tuo has dominant parameter on phosphate, Legon Cabe has dominant parameter on the atmospheric partial pressure of carbon dioxide (pCO₂), while Umang-umang Island has dominant parameter on carbonate minerals (calcite and aragonite). If we looked detail to the dominant parameter in Legon Tuo and Legon Cabe, phosphate and pCO₂, it has indicated any acidification process in both study sites. Compared with the result of pH, the concentration in Legon Tuo and Legon Cabe were lower than in Umang-umang Island. Therefore, Legon Tuo and Legon Cabe study site were suitable as acidification site, while Umang-umang Island as the reference site.

Table 1. Water quality in study site by direct measurement and carbonate chemistry calculated by CO₂SYS v2.1 (Pierrot et al., 2006)

Study site	Parameter				
	DO (ppm)*	pH*	Salinity (psu)*	Phosphate (ppm)*	Nitrat (ppm)
(1)	(2)	(3)	(4)	(5)	(6)
Legon Tuo	7.54 ± 0.33 ^a	8.17 ± 0.009 ^a	31.77 ± 0.06 ^a	0.82 ± 0.13 ^a	0.33 ± 0.14
Legon Cabe	7.56 ± 0.09 ^a	8.15 ± 0.006 ^a	31.57 ± 0.12 ^a	0.41 ± 0.08 ^b	0.31 ± 0.10
Umang-umang	6.93 ± 0.17 ^b	8.24 ± 0.010 ^b	34 ± 0.27 ^b	0.31 ± 0.15 ^b	0.44 ± 0.11

Study site	Parameter				
	Amonia (ppm)*	Alkalinity (mmol.kg ⁻¹ SW)*	pCO ₂ (µatm)*	Ω Calcite*	Ω Aragonite*
(7)	(8)	(9)	(10)	(12)	(13)
Legon Tuo	0.21 ± 0.01 ^a	2090.5 ± 3.6 ^a	250.48 ± 11.76 ^a	6.21 ± 0.15 ^a	4.11 ± 0.1 ^a
Legon Cabe	0.19 ± 0.01 ^a	2078 ± 7.20 ^a	262.90 ± 7.71 ^a	6.01 ± 0.11 ^a	3.98 ± 0.07 ^a
Umang-umang	0.28 ± 0.02 ^b	2231.7 ± 17 ^b	208.96 ± 10.92 ^b	7.60 ± 0.24 ^b	5.06 ± 0.16 ^b

Note: (*) means that the parameters have significant result to the study site ($p < 0.05$). Different alphabet in the same row indicate significant different ($p < 0.05$)

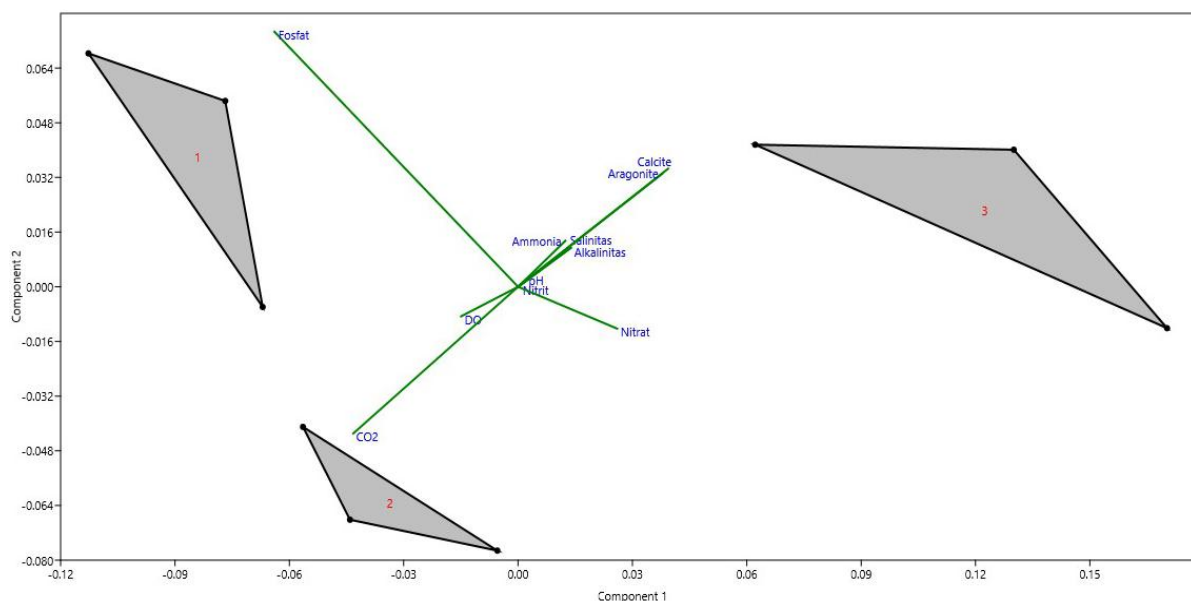


Figure 2. The relationship between water quality parameters with study site using canonical discriminant analysis (CDA), where 1, 2, and 3 indicate Legon Tuo, Legon Cabe, and Umang-umang island, respectively.

The results showed that the water quality indicated any acidification activity at the study site. According to multivariate analysis, Legon Tuo site has the highest phosphate concentration. This location is located close to Anak Krakatau volcano and has the hydrothermal vent from the inner part of the earth. The visibility showed more turbid compared to the other site during sampling. It can be an indication that area has high phosphate concentration. Meanwhile, pCO_2 has a dominant effect in Legon Cabe site that can be related to the position of study site which close to volcanic activity. The concentration of pCO_2 in Krakatau seas was 250.48-262.90 μatm , while in Umang-umang Island was 208.96. Increasing of pCO_2 could trigger the water become more acid (Hall-Spencer et al. 2008; Inoue et al., 2013).

If we looked at the pH concentration for the location near Krakatau and compared it to reference study site (Umang-umang Island), it would have less pH concentration until 0.08 units on average due to differences in pCO_2 concentration. Yet, it remained standing above 8. This condition was categorized as low acidification according to the previous study on Januar et al., (2016a). They conducted the study in three different sites near CO_2 seep (Minahasa, Gunung Api Island, and

Mahengetang Island) and divided the category of acidification into moderate (pH 7.8) and low (pH 8). In other studies, Hall-Spencer *et al.*, (2008) in Ischia Island, Italy, showed that pH concentration with pH 6.57-7.87. Compared to two previous cases, it indicated that pH in Krakatau seas has higher amount and categorized into low acidification. We thought the position of Krakatau islands in the middle of Sunda Strait with strong current velocity could be the reason low acidification in this area.

3.2. The potency of cytotoxic test in *Sarcophyton*

Extracts from our sample were tested to MCF-7 cell line to find a potential source of bioactive activity. **Figure 3** and **Table 2** showed the pictures of MTT assay during laboratory analysis and the result of growth inhibition percentage, respectively. We could find that samples which came from the acidification site, have higher growth inhibition percentage than the sample from the reference site. The mean of growth inhibition percentage from the acidification site was 74.55 %, while from the reference site was 21.57 %. Furthermore, these results tell us that samples from the acidification site were more active to inhibit the growth of MCF-7 cell.

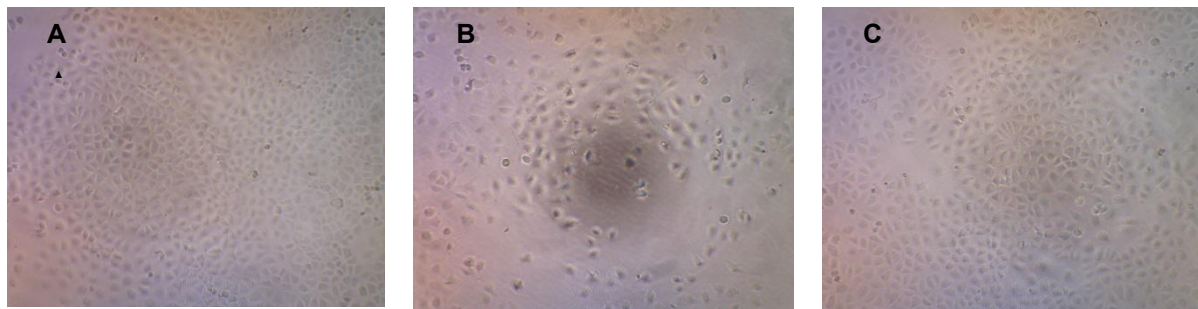


Figure 3. Cytotoxic test using the extract of *Sarcophyton* to MCF-7 cell (A) control, (B) sample from Legon Cabe, (C) sample from Umang-umang Island

Table 2. Growth inhibition percentage of *Sarcophyton* samples which were tested to MCF-7 cell line

Study site	Sample code	Growth inhibition of MCF-7 (%)
(1)	(2)	(3)
Legon Cabe (acidification)	BTN-78	49.09
	BTN-73	100
Umang-umang island (reference)	BTN-52	22.60
	BTN-104	6.09
	BTN-76	36.02

Samples from Krakatau have actively cytotoxic activity compared to samples from reference study site. The potency of bioactive compounds on soft coral were assumed due to the environmental condition. When declining on pH happened, soft coral will produce bioactive compound from metabolite activity. As the consequence, this condition will increase the ability to produce the bioactive compound to survive in unwanted condition. Although they can survive in the area with low pH, biological characteristic of soft coral could not increase on this condition. Januar et al., (2016a) on their research proved that cytotoxic activity was higher in the sample from pH 8 than the sample from pH 7.8. This indicated that soft corals have a threshold to tolerate in changing environment condition and influenced the potency of producing the bioactive compound.

Soft coral *Sarcophyton* was not dominant genus in Krakatau, yet in this study, cytotoxic activity showed the good result as an inhibitor of MCF-7 cell. In ecological condition, *Xenia* is the dominant genus in Krakatau. Putra et al., (2014) stated in Krakatau was dominant with *Xeniidae*. This condition different with the other acidification sites, such as Gunung Api Island (Maluku) and Mahengetang Island (North Sulawesi), was dominated by *Sarcophyton*.

This can be a potency for next research about cytotoxic activity from dominant soft coral in Krakatau.

Zubair et al., (2015) stated that there were 65 chemical compounds from eight species of soft coral *Sarcophyton* and have benefit as cytotoxic, antibacterial, antiviral, antidiabetic, anti-inflammatory, to neuroprotective activities. Iswani et al., (2014) stated soft coral *Sarcophyton* produced the cytotoxic compound, cembranoid sarcophytol, that active to MCF-7 cell line. Soft coral produces the cembranoid compound to survive in the ecosystem. If the ecosystem experienced high pressure condition, such as competition of space with hard coral and reef fish, *Sarcophyton* would increase in production of cembranoid, instead of lipid (Fluery et al., 2000).

Study about finding the new bioactive compound in soft coral need to be improved due to have much information that we can explore. Januar et al., (2016b) through their study in Mahengetang Island had successfully found a new cytotoxic cembranoid compound named 2-hydroxy-crassocolide E. According to their research, extract of *Sarcophyton* was effectively inhibited the growth of MCF-7 cancer, with 50% inhibition of tumor cell lines growth lower than 30 mg. L⁻¹. Therefore, these studies

tell us that there is big opportunity to find a potential source for new various bioactive compounds in soft coral species, especially for the organism that lived in extreme ecosystem.

4. Conclusion

The characteristic of Krakatau seas, especially in Legon Tuo and Legon Cabe study site, was categorized in low acidification. Soft coral *Sarcophyton* from Legon Cabe (acidification site) has actively inhibited the growth of breast cancer cell (MCF-7) than soft coral from Umang-umang Island (reference site).

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